Oral bacteria and colorectal cancer: Etiology and mechanism

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Background

Colorectal cancer (CRC) is the second leading cause of cancer death in men and women combined in the U.S., affecting one in every 20 individuals (1). Although it is considered a "Western" disease, the incidence is increasing worldwide. CRC has long been recognized as genetic disease, and follows an "adenoma-carcinoma" model developing as mutations accumulate (2). The genes most commonly mutated in CRC include adenomatous polyposis coli (*APC*), β-catenin (*CTNNB1*), *P53*, Kirsten rat sarcoma oncogene (*KRAS*), and myelocytomatosis oncogene (*MYC*) (3). These are driver mutations associated with several cancer hallmarks: uncontrolled cell growth and replication, resistance to apoptosis, angiogenesis, tissue invasion and metastasis, reprogramming of energy metabolism, evading immune destruction, and inflammation (4).

The advancement in microbial identification and human microbiome studies have revolutionized our view of the microorganisms associated with disease, including CRC. Using various "omics" approaches, a number of studies have consistently identified *Fusobacterium nucleatum* to be highly enriched in colorectal carcinomas (5–8). A subsequent study reported that *F. nucleatum* was not only enriched in CRC, but also in benign precancerous polyps (9).

F. nucleatum, a gram-negative anaerobe, is one of >600 microbial species inhabiting the human oral cavity, implicated in periodontal disease (10, 11). Increasing evidence suggests that oral bacteria are not confined to the oral cavity, and can migrate to extra-oral sites causing infections and inflammation (12). *F. nucleatum* has been isolated from a wide range of organ abscesses and infections, although it is never or rarely detected in those floras under normal conditions. For example, this organism is capable of crossing the placental barrier, causing pregnancy complications such as preterm birth, stillbirth and neonatal sepsis (12). Given that the oral cavity is at the beginning of the digestive tract, it is not surprising that microbial species find their way down the path. However, detection of *F. nucleatum* in the colorectal adenomas and carcinomas does not prove causality. Our study aims to address this issue and determine if *F. nucleatum* is indeed a driver of CRC (13).

Discussion

Our study reveals that FadA is a key virulence factor from *F. nucleatum*, which together with the genetic mutations, promote colorectal carcinogenesis (13). FadA is a novel adhesin identified and extensively characterized by our group (14–19). We have shown previously that FadA mediates *F. nucleatum* binding to endothelial and epithelial cells. It is highly

conserved among *F. nucleatum* but is absent in non-oral *Fusobacterium* spp (15). FadA binds vascular endothelial (VE)-cadherin on endothelial cells, causing increased endothelial cell permeability thus allowing bacteria to penetrate through, a likely mechanisms used by *F. nucleatum* for systemic dissemination (14).

In the current study, we showed that *F. nucleatum* binds and invades both normal and cancerous epithelial cells via FadA binding to E-cadherin. This binding leads to growth stimulation of human CRC cells but not the non-cancerous cells. FadA binding to E-cadherin on CRC cells causes nucleus translocation of β-catenin, thus activating β-catenin-regulated transcription (CRT), including increased expression of transcription factors LEF/TCF, oncogenes cyclin D1 and c-Myc, Wnt signaling genes Wnt 7a, 7b and 9a, and inflammatory genes NF-kappa B, IL-6, 8 and 18, all of which are hallmarks of carcinogenesis. Interestingly, although all these tumorigenic responses are activated by FadA through β-catenin, they are differentially regulated because the clathrin inhibitor, Pitstop 2, which inhibits *F. nucleatum* invasion but not its binding to the CRC cells, only inhibited the inflammatory responses such as NF-kappa B, IL-6, 8 and 18, but did not affect activation of the oncogenes or the Wnt signaling. Thus, activation of the inflammatory responses is invasion dependent, while activation of the oncogenes and Wnt signaling is not. A model of how FadA stimulates tumorigenesis is depicted in Fig. 1.

The FadA binding site on E-cadherin has been mapped to an 11-amino acid domain. A synthetic peptide corresponding to this domain prevents *F. nucleatum* from binding and invasion of CRC cells and blocked the activation of oncogenes, Wnt signaling and inflammatory responses (Fig. 1). It also inhibits *F. nucleatum*-driven CRC growth both *in vitro* and in xenograft mice.
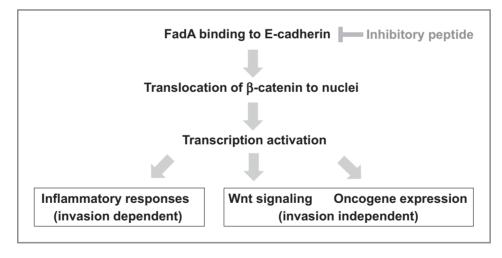

Fig. 1. Model of FadA from *F. nucleatum* activating tumorigenic responses. FadA binds to E-cadherin on colorectal cancer cells causing nucleus translocation of β-catenin, activating the inflammatory responses, Wnt signaling, and oncogene expression, all of which are hallmarks of carcinogenesis. Activation of the inflammatory responses is invasion dependent, while activation of Wnt signaling and oncogenes is not. The inhibitory peptide prevents FadA binding to E-cadherin and blocks all subsequence tumorigenic responses.

The *fadA* gene levels are significantly increased in the adenoma and carcinoma tissues, compared to the normal controls. The increase is step-wise, from normal controls to the precancerous state (including benign polyps and tissues surrounding the benign and malignant polyps), and from the precancerous state to carcinoma, with an average of 10-fold increase between each step. Furthermore, FadA transcription in *F. nucleatum* in the carcinoma tissue is significantly increased compared to that in the normal controls and the precancerous tissues, indicating an increase virulence activity of *F. nucleatum* in CRC. The increased FadA expression correlates with increased tumorigenesis responses in the carcinomas.

In a separate study, *F. nucleatum* was shown to induce tumor multiplicity and selectively recruit tumor-infiltrating myeloid cells to promote tumorigenesis in $APC^{+/-}$ mice. *Fusobacterium spp* were found to be enriched in adenomas and stools from adenoma and carcinoma patients (20). Together, these studies demonstrate that *F. nucleatum* stimulates colorectal carcinogenesis.

Future Directions

The discovery of an oral commensal, *F. nucleatum*, as a microbial driver of CRC provides a brand new perspective on the etiology, mechanism, diagnosis, treatment and prevention of this debilitating disease. The mechanistic studies identified novel diagnostic and therapeutic targets. Because it is unique to *F. nucleatum*, *fadA* may be an ideal diagnostic marker for early detection of CRC. Diagnostic criteria may be developed to define healthy, precancerous, and cancerous states according to the *fadA* gene levels. The inhibitory peptide and/or its derivatives may be used in precision medicine to specifically eradicate *F. nucleatum* to reduce CRC risk, similar to eradicating *H. pylori* to reduce gastric cancer risk. Compared to antibiotic therapies, the precision elimination avoids disturbance of the flora. The potential use of FadA in disease diagnosis, treatment and prevention warrants further testing.

Elucidation of the role of *F. nucleatum* in CRC sheds new light on the role of oral microbiome in human health. *F. nucleatum* is abundantly present in the oral cavity and increases in the presence of periodontal disease. Incidentally, both periodontal disease and CRC are considered "old people's diseases," with their risks increasing with age. Several questions thus arise: Is periodontal disease a risk factor for CRC? Given the connectivity of the digestive tract, could *F. nucleatum* or other oral bacteria be involved in additional GI disorders? Furthermore, based on the "mobility" of *F. nucleatum* and the omnipresence of cadherins, could this organism be involved in cancers beyond the GI tract? Answers to these questions will shed new lights on the role of oral microbiome in human health.

References

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